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AWARD NUMBER: W81XWH-08-1-0378

TITLE: Targeting IKK in Basal-Like Breast Tumors as a Theapeutic Approach

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REPORT DATE: June 2009

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
1 June 2009	Annual	1 June 2008 – 31 May 2009

4. TITLE AND SUBTITLE

Targeting IKK in Basal-Like Breast Tumors as a Theapeutic Approach

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-08-1-0378

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

University of North Carolina, Chapel Hill
Chapel Hill, NC 27599

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
Specifically, our hypothesis is that IKK and a form of NF-κB are activated in certain breast tumors (including the majority of basal-like tumors), leading to the expression of genes which promote oncogenesis and which lead to resistance to therapy. Additionally, we hypothesize that these tumors will respond to inhibitors of this pathway, either alone or in combination with chemotherapy. Based on our findings, we hypothesize that IKK/NF-κB and Bcl2A1 (a key gene regulated by NF-κB that is found upregulated in basal-like breast cancer) are key determinants of cancer therapy resistance in certain breast tumors. Our aims are to: (i) Generate a tumor bank archive for the analysis of NF-κB/IKK activation and associated gene expression, and correlate the findings derived from this analysis to breast tumor subtypes, (ii) Determine the mechanism of activation of Bcl2A1 and other NF-κB-dependent genes in basal-like cells; identify signaling components required for NF-κB activation in basal-like cancer cells; examine inhibitors of the NF-κB/IKK pathway in vitro, and (iii) Characterize animal models of breast cancer for activation of NF-κB and for potential therapeutic responses to NF-κB inhibitors.

15. SUBJECT TERMS
Breast cancer, NF-kappaB, IKK, animal models, drug studies

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			19b. TELEPHONE NUMBER *(include area code)*
U	U	U	UU	8	

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

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INTRODUCTION:

The goals of this grant are to determine if the NF-κB pathway is active in the basal-like breast cancer subtype and if this pathway can be targeted by small molecule inhibitors in a manner that is therapeutic. Patients with basal-like breast cancer typically exhibit poor outcomes, thus new therapies are required [refs. 1 – 5]. Our evidence is that a set of genes, known to be regulated by NF-κB, is upregulated in basal-like tumors and, interestingly, in cell lines that are basal-like. Some of the basal-like cell lines exhibit phosphorylated IKK, a key upstream regulator of the NF-κB pathway [6]. The NF-kB pathway is known to be involved in oncogenesis, but its role in basal-like breast cancer is unclear [refs. 7, 8]. Animal models of basal-like breast cancer also exhibit upregulation of some of these NF-κB-dependent genes. Our goals are to analyze basal-like cell lines, human tumors, and animal models of basal-like cancer to further validate our hypothesis that NF-κB is active in these tumors and may, therefore, represent a new therapeutic target for this breast cancer subtype with poor prognosis.

Aims ____ of the proposal are to: (i) analyze extracts of human breast cancer for phosphorylated IKK and other markers of NF-κB activation. Determine if these markers correlate with expression of Bcl2A1 and other NF-κB-dependent genes. (ii) determine the mechanism of activation of Bcl2A1 and other NF-κB-dependent genes in basal-like cancer cells, and compare this mechanism with pathways operative in distinct breast cancer subtypes (i.e., Her2+ cells). Analyze inhibitors of the NF-κB pathway for effects on growth and survival of basal-like, and other breast cancer cells. (iii). Analyze experimental tumors for markers outlined in Aims 1 and 2. Using animal models representative of basal-like and other breast cancers, determine if inhibitors analyzed in Aim 2 will suppress growth of the tumors, and/or sensitize the tumors to chemotherapy.

BODY (end of first year report):

Regarding Aim 1 goals:

--we have analyzed extracts of a number of breast tumors (7). We detected phosphorylated p65/RelA in samples 3, 4, 5, 6, and 7 (see Fig. 3). Bcl2A1 expression was found in tumor samples 2, 4 and 5. Tumors 2 and 4 are basal-like and 5 is luminal A/IIE subset (a tumor subtype that is known to express Bcl2A1). Thus, these results show that phospho-p65 ser536 is not directly correlated with Bcl2A1 expression, but that Bcl2A1 is expressed in 2/2 basal-like tumors (consistent with our hypothesis) but not in Her2+, luminal B, or luminal A (not IIE subtype).

Regarding Aim 2 goals:

--we have performed analysis of basal-like breast cancer cell lines and found the upregulation of the NF-κB subunit c-Rel (see Figs. 1 and 2). cRel is known to regulate Bcl2A1 in other cells.

--we performed analysis of Her2+ breast cancer cell lines which indicates that p65/RelA is phosphorylated, and that certain NF-κB-dependent genes are upregulated. Note that Bcl2A1 was not found in the Her2+ breast cancer cell lines, suggesting that either a different NF-κB subunit is involved in control of Bcl2A1 expression in basal-like cancer, or that a different cofactor is involved. IKKα and IKK β are both important in controlling gene expression and in activating NF-κB in these cells. IKKα drives invasion of these cells.

--treatment of basal-like breast cancer cell lines with the Bayer IKKβ inhibitor reduces expression of the associated NF-κB-dependent gene set and induces growth arrest (see Table 1 below).

Regarding Aim 3 goals, we have:

--Crossed the RelA fl/fl animal with Her2+ animals, along with expression of cre recombinase in the breast. This will test the role of the p65/RelA subunit in progression of Her2+ breast cancer.

--Begun treatment of the C3Tag animal model with our IKKβ inhibitor. The C3Tag animal is a model of basal-like cancer (it expresses genes found in human basal-like breast cancer).

KEY RESEARCH ACCOMPLISHMENTS:

--Analyzed markers for NF-κB/IKK activation and Bcl2A1 expression in human tumors.

--Contrasted Her2+ positive breast cancer cells with basal-like cells, indicating differential gene expression (and see manuscript described below).

--Treatment of basal-like breast cancer cells with an IKKβ inhibitor suppresses expression of the NF-κB-dependent gene set and induces growth arrest/apoptosis.

REPORTABLE OUTCOMES:

--Manuscript submitted regarding studies on Her2+ breast cancer cells and the involvement of IKK/NF-κB in controlling gene expression and invasion. This studies provides interesting parallels and differences with basal-like cancer.

--Evidence that c-Rel is upregulated in basal-like breast cancer cell lines.

CONCLUSIONS: Analysis of human breast tumor extracts confirms prediction that Bcl2A1 is active in basal-like cancers and in the luminal A IIE group. Analysis of basal-like breast cancer cell lines indicates that c-Rel is upregulated in these cells, which is a potential link with control of Bcl2A1 gene expression. Comparison of Her2+ breast cancer cells with basal-like cells indicates that NF-κB is active in both types of breast cancer, but that distinct genes are upregulated by NF-κB forms (potentially different subunits) in the two cancers. We have begun the proposed therapy studies in the model for basal-like cancer, using an IKKβ inhibitor which shows growth suppressive activity on basal-like breast cancer cell lines.

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APPENDIX

Figure Legends:

Fig. 1 (see first figure below). Nuclear extracts were generated from 3 basal-like breast cancer cell lines (Sum102, Sum149, MDA-MB-231), from a luminal-like breast cancer cell (MCF), and from a Her2+ breast cancer cell line (BT474). The nuclear extracts were used with a commercial gel shift/ELISA to determine nuclear levels of the 5 different NF-kB subunits. Results show that c-Rel and RelB are elevated in the basal-like cell lines.

Fig. 2 (see second figure below). Nuclear extracts from the Sum102 basal-like cells were treated with the IKKb inhibitor (Bay 65) and levels of c-Rel and RelB are diminished with treatment of Bay65.

Fig. 3 (see third figure below). Immunoblotting of whole cell extracts of 7 breast tumors stained with antibodies that recognize p65 phosphorylated at ser536, Bcl2A1 and tubulin. Tumors: 1 (Her2+), 2 and 4 (basal), 3 (luminal B), 5 (luminal A – IIE subset), 6 and 7 (luminal A). The results show that Bcl2A1 expression is detected in extracts of tumors from basal and IIE subsets. Phospho-p65 is detected but does not correlate with Bcl2A1 expression.

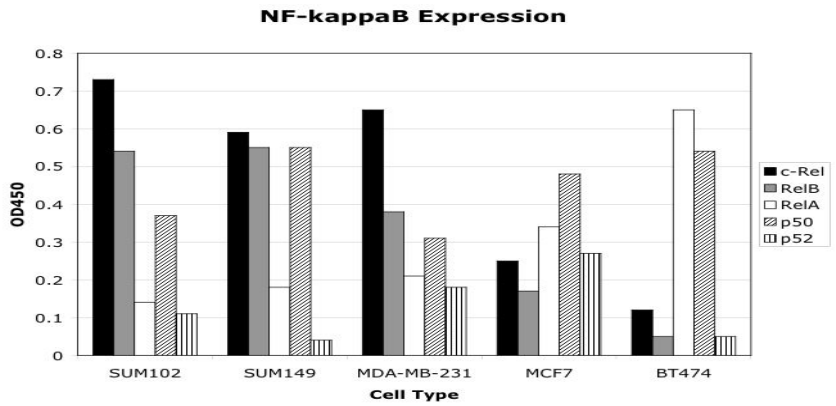

Fig. 6. "Gel shift" ELISA using 5 μg of nuclear extract from the indicated cell line. The NF-κB subunit tested is shown as different color bar graphs.

Fig. 1 (marked 6)

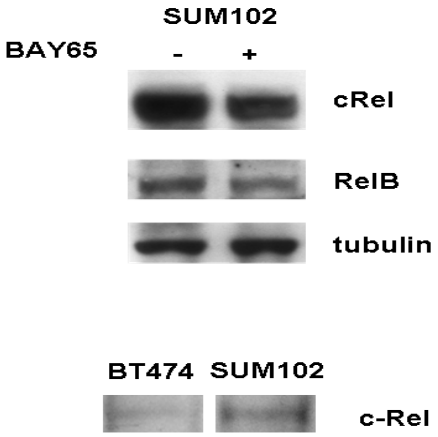

Fig. 7. Western blotting of nuclear extracts from SUM102 (top) with indicated antibodies (right), without or with Bay65 (1 μM for 4 hours). Bottom: c-Rel antibody on nuclear extracts from BT474 and SUM102 (light exposure).

Fig. 2 (marked 7)

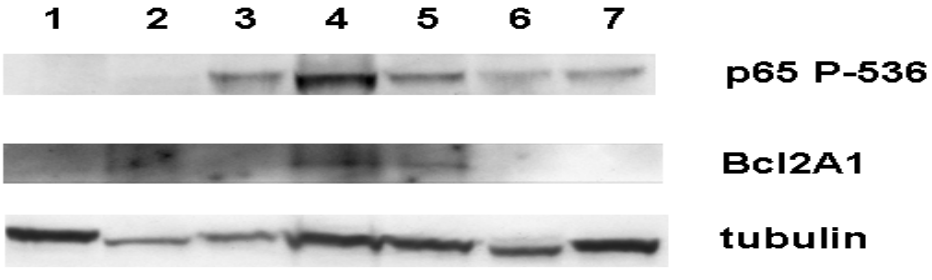

Fig. 3.

Table 1. Cell-Cycle Distribution of SUM102 cells treated with Bay65 (2.5 μM), a control (inactive) compound Bay 60, or with DMSO control. Results are an average of two experiments (less than 20% variation within the different phases).

	Cell-Cycle Phases		
	G0/G1	S	G2/M
Bay 65-1942	**87**	**6**	**7**
Control Bay cmpd	**58**	**26**	**16**
DMSO control	**55**	**21**	**24**